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*Long-Term Effects of Dredging Operations Program*

**Chronic Sublethal Effects of San Francisco  
Bay Sediments on *Nereis (Neanthes)*  
*arenaceodentata*; Bioaccumulation  
from Bedded Sediments**

by David W. Moore, Thomas M. Dillon  
Environmental Laboratory

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Prepared for Headquarters, U.S. Army Corps of Engineers  
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Interagency Field Verification of Methodologies for  
Evaluating Dredged Material Disposal Alternatives  
(Field Verification Program)



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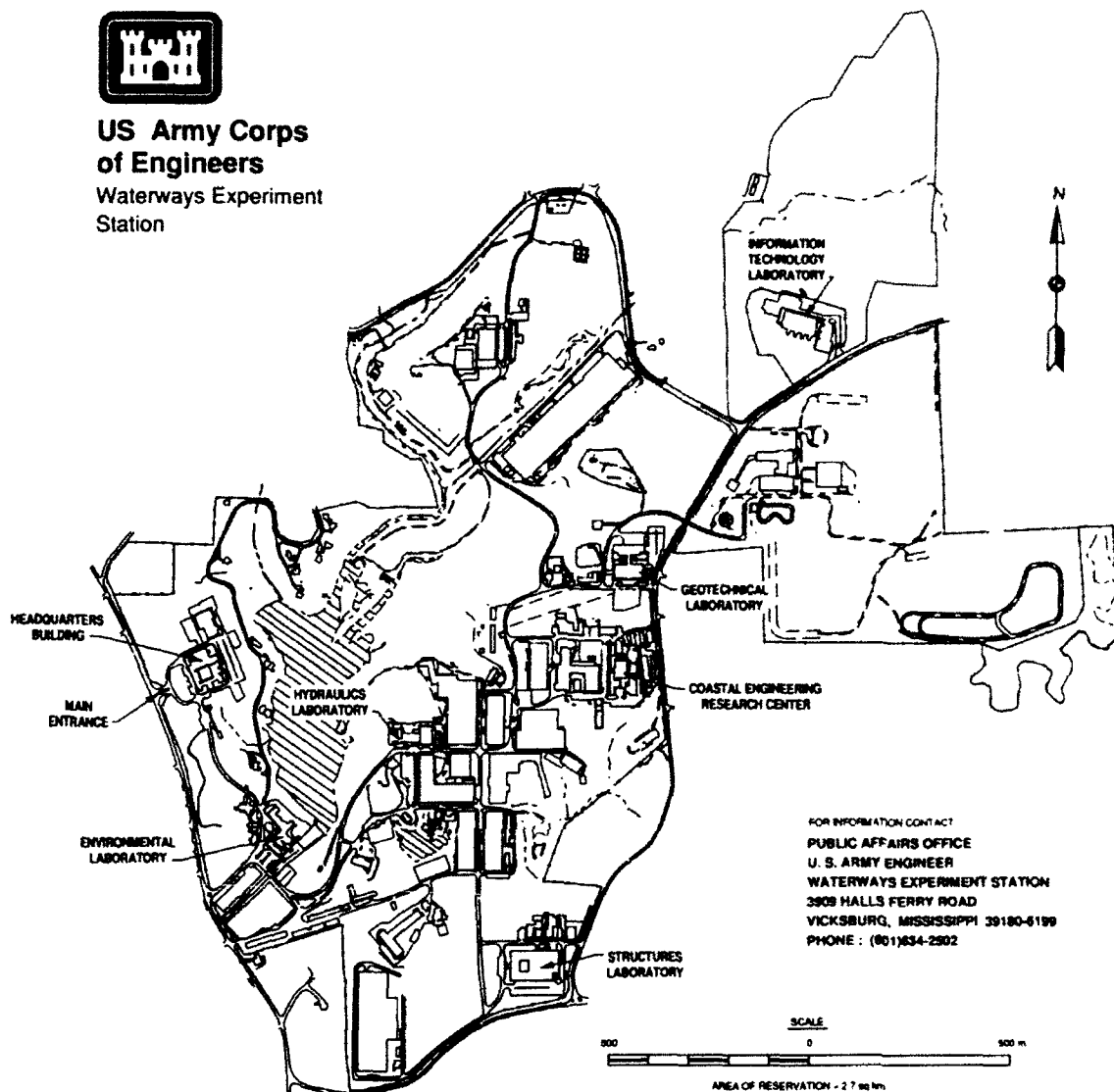
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# Preface

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The work reported herein was conducted by the U.S. Army Engineer Waterways Experiment Station (WES) for Headquarters, U.S. Army Corps of Engineers (HQUSACE), and the U.S. Army Engineer District (USAED), San Francisco. Financial support was provided by the USAED, San Francisco, through an Intra-Army Order for Reimbursable Services. Additional funding was provided by HQUSACE through the Long-Term Effects of Dredging Operations (LEDO) Program, Work Unit 374-9, "Chronic Sublethal Effects." The LEDO Program is managed through the Environmental Effects of Dredging Programs, Dr. R. M. Engler, Manager.

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# 1 Introduction

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## Background

San Francisco Bay is a highly altered estuary. Two of the major reasons for this condition are the diversion of freshwater inflow from the Sacramento-San Joaquin River systems and the loss of wetlands. As of 1980, nearly 60 percent of the historic freshwater inflow to San Francisco Bay estuary had been diverted, mostly for agricultural irrigation. This reduction is projected to increase an additional 10 percent by the year 2000. About 95 percent of all freshwater/estuarine marshlands have been lost to land reclamation since 1850. It is not surprising, therefore, that the estuary has experienced a general decline in health and viability. One of the more noticeable symptoms of this decline has been the gradual loss of biological resources such as the striped bass and Pacific herring fisheries (Nichols et al. 1986).

An increase in the input of environmental contaminants has accompanied the physical alterations to San Francisco Bay. Major pollutant sources include metals associated with mining tailings located in Sacramento-San Joaquin River drainage basins. Additionally, over 50 waste treatment plants and about 200 industries are permitted to discharge directly into the Bay (Luoma and Phillips 1988). Environmental contaminants discharged into aqueous systems tend to associate with particulate material in the water column and with bedded sediments. Periodically, bedded sediments must be removed to maintain navigable waterways. There is concern that the relocation of these dredged materials may be causing unacceptable adverse impacts on aquatic biota within San Francisco Bay.

A large amount of sediment is dredged each year in San Francisco Bay. Approximately 5.5 million cubic meters (mcm) of sediment from Federal projects and permit actions are relocated annually. This value approximates the estimated average annual sediment inflow from natural sources of 6 to 8 mcm (U.S. Army Corps of Engineers (USACE) 1979). It has been estimated that 3.0 to 4.0 mcm of material leaves the Bay annually, while Central and North Bays experience a combined net accumulation of 4.2 mcm (USACE 1979). South Bay shows a net loss of nearly 0.8 mcm per year (Krone 1979). Despite these large numbers, the greatest yearly source of suspended sediment in San Francisco Bay is the resuspension of existing bottom material. Approximately 120 to 130 mcm of sediment are resuspended each year by

wind waves and currents (USACE 1979). The effect of these resuspended sediments on fish and aquatic invertebrates is unknown.

To examine whether San Francisco Bay dredged material was causing adverse biological effects, the Planning and Engineering Division of the U.S. Army Engineer District, San Francisco, contracted with the Environmental Laboratory of the U.S. Army Engineer Waterways Experiment Station (WES) to develop and conduct a series of chronic sublethal sediment bioassays using material from selected sites within the Bay.

## **Regulatory History of Dredged Material Management in San Francisco Bay**

To help define what is known regarding the potential toxicity of San Francisco Bay sediments, it is useful to first examine how dredged material has been regulated in the past. Important milestones in that process are shown in Table 1. It was recognized very early that San Francisco Bay is a physically dynamic system and that most dredged material disposal sites were dispersive. Consequently, initial management concerns were mostly operational. That is, efforts were directed towards optimizing dredging and disposal operations to minimize transportation costs and redredging.

Passage of the National Environmental Policy Act in 1970 outlined the Federal Government's policy toward the environment and signaled an increasing desire for environmental protection in this country. That same year, the San Francisco District initiated the Dredge Disposal Study (DDS) (USACE 1977). The DDS was a multifaceted interdisciplinary study designed, in part, to address some of the environmental concerns regarding potential impacts of dredge disposal operations. Although sediment toxicity was not examined directly, the physical impacts on biota (USACE 1975a) and the bioaccumulation of contaminants from dredged material were evaluated in laboratory and field studies (USACE 1975a,b). Those studies demonstrated the following:

- a. Estuarine animals can survive suspended sediments loads in excess of those normally encountered during dredging and disposal.
- b. In laboratory exposures to San Francisco Bay sediments, estuarine animals can bioaccumulate trace contaminants.
- c. In field studies, contaminant tissue concentrations in animals near the disposal operations were not different from those far removed. The one exception was slightly elevated p,p'-DDE concentrations in mussels, *Mytilus edulis*, during disposal. These differences were not detected 1 month postdisposal.

In 1972, the California Regional Water Quality Control Board (RWQCB) adopted the Jensen criteria (Bowden 1977). These numerical criteria were



developed by the U.S. Environmental Protection Agency (USEPA) for fresh-water sediment in the Great Lakes and classified sediment as highly polluted, moderately polluted, or slightly polluted based on bulk sediment chemistry. As research on dredged material progressed, it became clear that these and other chemically based numerical criteria were technically inadequate because they did not assess either bioaccumulation potential or toxicity.

The San Francisco District adopted the use of bioassays for evaluating dredged material in 1980. Regulatory procedures were outlined in Public Notice (PN) 78-1. Elutriate procedures were emphasized since disposal sites in San Francisco Bay were generally dispersive. PN 78-1 also reduced the number of disposal sites from 5 to 3. These were located in the Carquinez Strait, San Pablo Bay, and near Alcatraz Island. To facilitate net export out of the Bay, most dredged material was taken to the Alcatraz disposal site.

In 1982, shoaling was noted at the Alcatraz site. As a result of this important development, the San Francisco District took several steps. The District instituted a slurry policy to enhance dispersion during disposal. They greatly reduced the amount of new dredged material taken to the Alcatraz site and even removed 30 tons (27,200 kg) of construction debris from the site. They monitored the physical configuration of the mound at Alcatraz and found it to be stable after two winter seasons. All of these actions led to the conclusion that the Alcatraz site could not be considered fully dispersive. Since the majority of dredged material in San Francisco Bay was taken to Alcatraz, a reduction in the capacity of that site represented a major impediment to maintenance dredging and to anticipated new work activities. The San Francisco District established the Disposal Management Program (DMP) in 1985 and charged it with finding solutions to the disposal problem.

The Long-Term Management Strategy (LTMS) was initiated in 1989 to address increasing environmental concerns and to reflect the San Francisco District's commitment to an LTMS for dredged material. In 1991, the Ocean Disposal Implementation Manual was revised to reflect 14 years of regulatory experience and the many scientific advances that had occurred since 1977 (USEPA/USACE 1991).

## **Overview of Sediment Toxicity Test Development in the United States**

As indicated in the foregoing discussion, the regulation of dredged material disposal in San Francisco Bay has taken advantage of scientific advancements that have occurred elsewhere in the United States. To address concerns specific to the potential toxicity of San Francisco Bay sediments, it is important to have some general knowledge of advances in the field of sediment ecotoxicology. The following is not intended to be a comprehensive review per se; rather it is meant to provide the reader with an overview of the advances that have occurred over the past 20 years.

The first peer-reviewed journal article that reported an attempt to assess sediment toxicity was published in 1971 (Cannon and Beeton 1971) (Table 2). The laboratory procedure involved exposing amphipods to freshwater dredged material that had been placed in modified milk cartons. In 1973, recognizing the need for a strong technical base in its regulatory program, the USACE initiated the Dredged Material Research Program (DMRP). Included in the scope of this large program was the development of elutriate and solid phase bioassays to assess potential water column and benthic impacts, respectively (Saucier, Calhoun, and Engler 1978). The bioassays developed during the DMRP were subsequently incorporated into both the Ocean Disposal Implementation Manual (USEPA/USACE 1977) and the interim guidance manual for discharge of dredged or fill material into navigable waters (i.e., the 404 manual) (USACE 1976). These sediment bioassays represented a balance between the state of the art and what could be routinely conducted in a regulatory program.

Prior to the mid-1970s, the scientific community expressed relatively little interest in sediment toxicity. Most of their energies were focused on the fate and effects of environmental contaminants dissolved in aqueous solutions. After the Priority Pollutant List was published in 1976, emphasis shifted for two reasons. One, it was discovered that many chemicals on the Priority Pollutant List were not very soluble in water and, hence, were not bioavailable. Two, as more field data were gathered, it became apparent that concentrations of many contaminants of the Priority Pollutant List were much higher in the sediment than in the overlying water. Those findings led to initial speculation that sediments might be extremely toxic. However, subsequent research showed that the same forces causing chemicals to partition into the sediments also restricted their bioavailability to aquatic organisms.

A major milestone marking these scientific advances was the 6th Pellston Conference held in 1984 (Dickson, Maki, and Brungs 1984). This was the first time leaders in the scientific community formally met to discuss the fate and effects of sediment-associated contaminants. Bioassay procedures contained in the 1977 USEPA/USACE Ocean Disposal Implementation Manual formed the basis for initial discussion. The researchers reached consensus regarding sediment toxicity (Anderson et al. 1984). They recognized that species sensitivity was related, in part, to the degree of contact between sediment and organism. They recommended amphipods and mysid shrimp for lethal tests polychaetes, bivalves, oligochaetes, and fish for behavioral or sublethal tests. There was also a strong endorsement of the Tiered Testing Approach for evaluating contaminated sediments (USEPA/USACE 1991). This approach eliminates unnecessary testing and directs limited resources to solving more urgent problems.

Another important milestone in the evolution of sediment toxicity methods occurred in 1987. Members of the American Society for Testing and Materials (ASTM) created a new Subcommittee, E47.01 Sediment Toxicology. This Subcommittee was charged with identifying technically sound procedures for evaluating sediment toxicity and with drafting appropriate

standardized guideline documents. Guidelines, which are in various states of preparation include the following:

- a. Solid Phase Toxicity Tests with Freshwater Invertebrates.
- b. Solid Phase Toxicity Tests with Marine Amphipods.
- c. Solid Phase Toxicity Tests with Marine Polychaetes.
- d. Solid Phase Bioaccumulation Tests with Invertebrates.
- e. Solid Phase Bioaccumulation Tests with Fish.
- f. Guidance for Designing Sediment Toxicity Tests.
- g. Guidance for Collection, Storage, Characterization, and Manipulation of Sediment Prior to Toxicity Testing.

When the USEPA/USACE Ocean Disposal Implementation Manual was first published in 1977, the procedures it contained represented a balance between the state of the art and what could be achieved in the regulatory testing environment. It was realized at that time that revisions would have to be made to reflect scientific and regulatory advances. The Manual was revised in 1991. Significant improvements to the current Manual, as related to sediment toxicity evaluation, include the following:

- a. Formalizing the Tiered Testing Approach.
- b. Refinements to the species selection process.
- c. Provisions for evaluating chronic sublethal effects.

The assessment of chronic sublethal effects is treated as a Tier IV assessment and would be carried out only if there is a reason to believe chronic impacts may be occurring and if technically sound test protocols are available.

## Scope

The objective of this report is to assess potential bioavailability of contaminants from a bedded San Francisco Bay sediment. This study is part of a larger effort to evaluate the chronic sublethal toxicity of San Francisco Bay sediments. This report is not designed to be used in a regulatory decision-making process (i.e., 404 or 103), but rather is intended to provide input to the District's Disposal Management Plan and LTMS for dredged material disposal in the San Francisco Bay area.

In two previous reports (Moore and Dillon 1993; Moore and Dillon, In Preparation), survival, growth, and reproduction in *N. arenaceodentata* were

evaluated in both partial (i.e., from the emergent juvenile stage through pairing of sexually mature adults) and full life-cycle (i.e., from the emergent juvenile stage through production of a second generation) exposures to San Francisco Bay sediments. Bulk chemical analysis of the sediment indicated metal and polycyclic aromatic hydrocarbon (PAH) contamination in a few of the sediments tested. However, only minimal chronic sublethal effects were observed in either test. One potential explanation for this apparent lack of effect would be a lack of contaminant uptake by the test organism. To address this question, a bioaccumulation experiment was performed in which tissue residues of animals exposed to a San Francisco Bay sediment known to be contaminated were compared with residues of animals reared in a "clean" control sediment. Results were used to verify contaminant uptake by animals exposed to these same sediments in previous chronic sublethal experiments.

## 2 Material and Methods

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### Test Species

*Nereis (Neanthes) arenaceodentata* is a benthic infaunal polychaete widely distributed in shallow marine and estuarine benthic habitats of Europe, all three coasts of North America, and the Pacific (Reish 1957, 1963; Sanders et al. 1962; Pettibone 1963; Reish and Alosi 1968; Day 1973; Gardiner 1975; Whitlatch 1977; Taylor 1984). This subsurface deposit-feeder constructs one or more mucoid tubes in the upper 2 to 3 cm of sediment and ingests sediment particles up to 70  $\mu\text{m}$  with a preference for particles around 12  $\mu\text{m}$  (Whitlatch 1980). *Nereis (Neanthes) arenaceodentata* has been accepted by the regulatory community as an appropriate test species for evaluating sediment (USEPA/USACE 1977, 1991; Johns, Gutjahr-Gobell, and Schauer 1985). A considerable amount of toxicological information on a wide variety of environmental contaminants already exists for this species (Reish 1985; Jenkins and Mason 1988; Anderson et al. 1990).

Taxonomists are still debating the appropriate nomenclature for this species. Pettibone (1963), who sugges. *J. N. arenaceodentata*, lists five names in synonymy for this species: *Spio caudatus*, *Nereis (Neanthes) caudata*, *Nereis arenaceodentata*, *Neanthes cricognatha*, and *Neanthes caudata*. Day (1973) dismissed *arenaceodentata* in favor of *acuminata*, which was subsequently used by Gardiner (1975), Taylor (1984), and Weinberg et al. (1990). *Neanthes arenaceodentata* is most commonly used in the toxicological literature. Recent evidence suggests that Atlantic and Pacific populations are genetically dissimilar, reproductively isolated, and probably different species (Weinberg et al. 1990). Until the taxonomic status of this species is resolved, the name most familiar to toxicologists will be used and the original source of worms reported.

The life cycle of *N. arenaceodentata* is well documented, as are culture methods (Reish 1980). As worms approach sexual maturity, males and females establish pairs and occupy a common tube. Eggs are deposited by the female within the tube, and the male presumably fertilizes the eggs at this time. The spent female either exits the tube and dies within 1 to 2 days or is eaten by the male. The male remains in the tube to incubate and guard the developing eggs. He creates a current of water via rhythmic undulations to remove metabolic waste and prevent hypoxic conditions.

Larval development is direct via nonplanktonic metatrochophore larvae and occurs entirely within the parental tube. Emergent juveniles (EJs) exit the parental tube about 3 weeks after egg deposition. After emergence, juveniles begin to feed and establish tubes of their own. Juvenile worms grow and eggs become visible in the coelom of females about 6 weeks postemergence. Egg deposition follows 3 to 7 weeks later. The entire life cycle can be completed in the laboratory in 12 to 16 weeks at 20 to 22 °C. Nonplanktonic benthic larvae and paternal care are unique among the Nereidae. These features also facilitate laboratory culture and the experimental investigation of sublethal effects on growth and reproduction.

## Laboratory Cultures

Stock populations of *N. arenaceodentata* were obtained in March 1988 from Dr. D. J. Reish, California State University at Long Beach. Laboratory cultures were maintained using methods adapted from those described by Reish (1980) and Pesch and Schauer (1988).

Briefly, EJs were raised to sexual maturity in 38-L aquaria containing 30 L of 30-ppt seawater (Instant Ocean) maintained at a temperature of 20 °C. The photoperiod was 12 hr light. Animals were fed a combination of ground Tetramarin flakes (1 mg/worm) and alfalfa (0.5 mg/worm twice weekly. This feeding regime is sufficient to maintain adequate water quality in a static-renewal system and has been found to produce survival and reproduction consistent with what has been reported for other laboratory populations of *N. arenaceodentata* (i.e., survival > 80 percent; fecundity, ca. 100 to 1,000 eggs/brood; EJ production, ca. 50 to 500 EJs/brood) (Reish 1980; Pesch et al. 1987; Anderson et al. 1990).

Seawater was renewed (80 percent of volume) every 3 weeks. This renewal schedule, based on water-quality monitoring data, is sufficient to maintain good water quality. After 10 weeks, worms were paired using the fighting response (Reish and Alosi 1968) and the presence or absence of eggs in the coelom. Unpaired worms were discarded. Pairs were placed in 600-ml beakers with 500 ml of seawater. Gentle aeration was provided via Pasteur pipettes, and the beakers were covered with watch glasses to reduce evaporation. Water was carefully renewed weekly in a manner that avoids disturbing worm pairs.

Beakers were monitored daily for the presence of eggs and EJs. Discovered EJs were mixed with EJs from other broods and returned to the 37-L aquaria to complete the culture cycle. These culture conditions and feeding rations were used in the experiment described below unless otherwise noted.

## Test Sediments

Sediments fell into two categories: test sediment (i.e., a San Francisco Bay sediment known to be contaminated) and a control sediment (used in cultures of the test organism). Previous studies evaluating the chronic sublethal toxicity of San Francisco Bay sediments found only minimal effects (Moore and Dillon 1993; Moore and Dillon, In Preparation). Selection of the test sediment for this study was based solely on sediment chemistry and the number and magnitude of contaminants observed. The test sediment was a composite of several cores taken to project depth (38 ft (11.6 m) below mean low water mark) from areas of Oakland Inner Harbor known to be contaminated. For purposes of this report, this sediment will be designated as the Oakland Contaminated (OC) sediment. A control sediment from Sequim, WA, was also tested. The Sequim Control (SC) sediment was essentially free of contamination and was used to evaluate experimental results. Sediment collection was performed under the direction of Battelle Pacific Northwest Laboratory (for a complete description of sampling methods and protocols, see Mayhew et al., In Preparation). Coordinates for sampling locations may be found in Appendix A.

Sediment samples were immediately refrigerated (4 °C) on collection and shipped via a refrigerated truck to WES. Upon receipt at WES, sediment samples were wet sieved (< 2 mm), thoroughly homogenized, and refrigerated (4 °C) until analysis and testing could be performed. Three composites from each of the two sediments were analyzed for priority pollutant metals (except antimony and thallium), chlorinated pesticides, polychlorinated biphenyls (PCBs), and PAHs. Analysis was performed by the Analytical Laboratory Group (ALG) at WES according to procedures outlined in USEPA SW-846 (USEPA 1986). Sediments were also analyzed for tributyltins, dibutyltins, and monobutyltins (TBT, DBT, and MBT) by the Naval Command and Control and Ocean Surveillance Center (NRCOSC) in San Diego, CA, using procedures outlined by Stallard, Cola, and Dooley (1989). Total organic carbon (TOC) and Total Kjeldahl nitrogen (TKN) analyses were performed by the ALG using Standard Method 505c (Standard Methods for the Examination of Water and Wastewater 1989) and procedures outlined in USEPA (1979), respectively. Grain size analysis was performed using the methods of Patrick (1958). Percent loss of volatile solids after ignition at 550 °C (LOI) was determined using Standard Methods 209a and 209c (Standard Methods for the Examination of Water and Wastewater 1989). In addition, pore water was extracted from each of the sediments using methods described by Ankley, Katko, and Arthur (1990). Sediment pore water extracts were subsequently analyzed for total ammonia ( $\text{NH}_3$ ) and hydrogen sulfide ( $\text{H}_2\text{S}$ ). Samples for ammonia analysis were adjusted to a pH of 2 with 1 N hydrochloric acid (HCL) and stored at 4 °C for no longer than 2 weeks. Total ammonia (milligrams/liter) was determined with an Orion ammonia-specific electrode after adjusting sample pH to 12 with 5 N NaOH. Pore water extracts were analyzed for  $\text{H}_2\text{S}$  using a HACH HS-7 test kit. This kit makes use of the color reaction between lead acetate and hydrogen sulfide. Filter pads impregnated with lead acetate are exposed to effervescing water samples containing hydrogen sulfide. The ensuing color change in the filter pad is compared

with a standardized chart accompanying the kit to yield a semiquantitative measurement of hydrogen sulfide. Additional information on detection limits, instrumentation, and quality assurance protocols for analyses performed by the ALG can be found in Strong and Myers (1991).

## Experimental Approach

Bioaccumulation from both the SC and OC sediments were evaluated in a 9-week exposure with the marine polychaete *N. arenaceodentata*. Sediments were added to 120-L, aged Fiberglass tanks to a depth of 2.5 cm. Eighty liters of 30-ppt salinity seawater was gently added to each tank, carefully avoiding resuspension of the bedded sediment. To initiate the test, emergent juvenile worms ( $n = 1,800$ ) were taken from laboratory culture and randomly distributed among six tanks. There were three tanks/treatment and 300 EJs/tank. This stocking density has been found to provide optimal growth and survival of *N. arenaceodentata*. The test was conducted under static-renewal conditions (renewal every 3 weeks) at an ambient laboratory temperature of  $20 \pm 2$  °C and a 12-hr photoperiod. Gentle aeration was provided to each tank. Worms were fed twice weekly a combination of finely ground Tetramarin and alfalfa prepared in a seawater slurry. Worms were exposed to test sediments for 9 weeks. Dissolved oxygen, salinity, temperature, and pH were monitored weekly. In addition, a 30-ml sample was collected from each aquarium, fixed with 50  $\mu$ l of 1 N HCL, refrigerated, and subsequently analyzed for total ammonia by methods previously described for analysis of total ammonia in sediment pore water.

After 9 weeks, worms were removed from all tanks. Worms recovered from each tank were placed in 1-L glass culture bowls containing clean 30-ppt salinity seawater under aeration and allowed to purge their gut contents for 24 hr. Worms were then placed into 100-ml polycarbonate sample bottles and frozen for subsequent tissue residue analysis. Tissues were analyzed for priority pollutant metals (except antimony and thallium), chlorinated pesticides and PCBs, and PAHs. Analyses were performed by the ALG at WES according to procedures outlined in USEPA SW-846 (USEPA 1986). Tissues were also analyzed for tributyltins, dibutyltins and monobutyltins by the NRd in San Diego, CA, using procedures outlined by Stallard, Cola, and Dooley (1989).

## Statistical Analysis

All statistical analysis and data transformation were conducted using SYSTAT statistical software (Wilkinson 1988). All data were screened for homogeneity of variance prior to statistical analysis via Bartlett's test. Results were compared using a pooled t-test (Sokal and Rohlf 1981). All tests for significance were analyzed at a significance level of  $\alpha = 0.05$ .



## 3 Results

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### Test Sediment

Results of physicochemical analysis of the SC and OC sediments are summarized in Table 3. Grain size analysis indicated that the SC and OC sediments were fine grained (ie., mostly silt and clay). Both sediments were relatively similar in terms of moisture content with mean values of 64.7 and 51.0 percent for the SC and OC sediment, respectively. Mean percent LOI and TOC were significantly higher in the SC sediment (percent LOI = 14.0, percent TOC = 0.69) relative to the OC sediment (percent LOI = 11.8, percent TOC = 0.21). Similarly, mean TKN, a measure of potential nutritional value, was significantly higher in the SC sediment (3,540 mg/kg) relative to the OC sediment (553 mg/kg). Analysis of sediment pore water extracts also showed marked differences between sediment types. Mean pore water total ammonia levels were significantly lower in the SC sediment (13.7 mg/L) compared with the OC sediment (42.2 mg/L). Conversely, mean pore water hydrogen sulfide levels were significantly higher in the SC sediment (133 mg/L) relative to the OC sediment (<0.01 mg/L).

Results of chemical analyses for each of the sediment types suggest a common trend. Concentrations of metals and PAHs were significantly higher in the OC sediment when compared with the SC sediment. Specifically, concentrations of the metals chromium, copper, lead, mercury, nickel, and zinc (measured in the milligram/kilogram range) were significantly higher in the OC sediment relative to the SC sediment (Table 4). Additionally, silver and butyltins, measured in microgram/kilogram quantities, were significantly higher in the OC compared with the SC sediment. Selenium, also measured in microgram/kilogram quantities, was the only metal found at significantly higher concentrations in the SC sediment relative to the OC sediment (Table 4). PAHs were not detected in the SC sediment, while phenanthrene, fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene, and benzo(g,h,i)perylene were all measured in the milligram/kilogram range in the OC sediment (Table 5). With the exception of a small amount (microgram/kilogram range) of p,p'-DDE detected in a single replicate of the OC sediment, no significant concentrations of pesticides or PCBs were found in either of the sediments tested (Table 6).

## **Tissue Residues**

Analysis of tissue residues following 9 weeks exposure to the test sediments indicated some bioaccumulation of metals and pesticides. Results of metal analyses indicated significantly higher concentrations of silver and tributyltin in worms exposed to OC sediment compared with worms exposed to SC sediment (Table 7). Conversely, concentrations of cadmium and lead were significantly higher in animals exposed to the SC sediment compared with animals exposed to the OC sediment. Though not statistically different, levels of chromium and zinc were also measured at higher concentrations in worms exposed to SC sediment relative to those exposed to the OC sediment. Because of a limited amount of tissue, samples for PAH, pesticide, and PCB analysis were pooled. PAHs were not detected in worms exposed to either sediment (Table 8). Results of pesticide analysis indicated microgram/kilogram quantities of aldrin in worms exposed to OC sediment,  $\delta$ -BHC in worms exposed to SC sediments, and dieldrin in worms recovered from both the SC and OC sediments (Table 9). PCBs were not detected (Table 9).

## **Water Quality**

Water quality was good in all sediment exposures (Appendix B.)

## 4 Discussion

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Nereid worms have been shown to be good organisms for bioaccumulation studies (Rubinstein et al. 1990). Both *N. arenaceodentata* and *Nereis virens* are recommended for the regulatory evaluation of sediment bioaccumulation potential (USEPA/USACE 1991). However, a comparison of tissue residue data from worms exposed to a contaminated sediment from San Francisco Bay and a "clean" control sediment indicates little bioaccumulation of contaminants by the marine polychaete worm *N. arenaceodentata*. The minimal bioaccumulation observed in this study may account in part for the minimal chronic sublethal toxicity observed in earlier studies with *N. arenaceodentata* exposed to the same sediments (Moore and Dillon 1993; Moore and Dillon, In Preparation).

While sediment concentrations of a number of metals and PAHs in the OC sediment were significantly higher than the SC control sediment, similar trends were not apparent in the tissue residue data. PAHs were not detected in worms recovered from either sediment. McElory (1990) has shown rapid metabolism of PAHs via a cytochrome P-450 enzyme system in another nereid polychaete, *Nereis virens*. If *N. arenaceodentata* possessed a similar ability, the PAHs may have been metabolized too rapidly for bioaccumulation to occur. Of the metals, only silver and tributyltin were significantly higher in the tissues of animals recovered from OC sediment relative to the SC sediment. A number of metals (i.e., cadmium, chromium, lead, and zinc) were found in higher concentrations in the control animals. This was surprising since the concentrations of cadmium were not significantly different in the two sediment types and concentrations of chromium, lead, and zinc were significantly higher in the OC sediment relative to the SC sediment. While this anomalous response cannot be accounted for, several speculations can be offered. One potential explanation would be contaminated seawater and/or food supply. Since both groups of animals were exposed to the same source of seawater and food, this explanation is not plausible. Another explanation might be that differences in acid volatile sulfides (AVS) between the sediment types affected bioavailability. AVS have been shown to affect metal bioavailability in sediment (i.e., at higher AVS concentrations, metal are less bioavailable) (Carlson et al 1991; Di Toro et al. 1992). While AVS was not measured directly, the high pore water concentrations of  $H_2S$  observed in the SC sediment (133 mg/kg) relative to the OC sediment (<0.01 mg/kg) suggest that AVS was higher in the SC sediment and, consequently, metals should be less bioavailable. Perhaps a more likely explanation is that factors other than

AVS affected metal bioavailability and uptake. For example, some metals (e.g., cadmium, chromium, lead, and zinc) may have been in the form of filings or shavings in the OC sediment and, therefore, not available for uptake by the organism. In addition, animals were fed during the study. Therefore, it is possible that the worms in the OC sediment reduced exposure by feeding preferentially on the external food source to the exclusion of the test sediment. During the test, a greater number of tubes on the sediment surface were noted in the OC sediment compared with the controls. This suggests that the animals may have avoided contact with the OC sediment.

The minimal toxicity observed in earlier studies with OC sediment appears to be explained by a lack of contaminant uptake. Only tributyltin and silver were observed at levels significantly higher than control. In addition, the mean tissue concentration of tributyltin measured in this study (0.298 mg/kg) was well below levels shown to exert chronic sublethal toxicity (i.e., > 3 mg/kg) in *N. arenaceodentata* (Moore, Dillon, and Suedel 1991). While Pesch and Hoffman (1983) has evaluated the chronic sublethal toxicity of silver in this species, corresponding residue effects information is not available. Consequently, the potential toxicity of the silver tissue residues reported herein cannot be evaluated.

## 5 Conclusions

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*Nereis (Neanthes) arenaceodentata* were exposed for 9 weeks to a sediment from San Francisco Bay known to be contaminated with metals and PAHs. Conclusions based on this study are summarized below.

- *Nereis (Neanthes) arenaceodentata* exposed to a contaminated San Francisco Bay sediment accumulated significant amounts of tributyltin (0.298 mg/kg dry weight) and silver (0.30 mg/kg dry weight).
- *Nereis (Neanthes) arenaceodentata* exposed to a contaminated San Francisco Bay sediment accumulated small amounts of aldrin and dieldrin (i.e., 0.016 and 0.021 mg/kg dry weight, respectively).
- *Nereis (Neanthes) arenaceodentata* exposed to a contaminated San Francisco Bay sediment did not accumulate polycyclic aromatic hydrocarbons or polychlorinated biphenyls.
- *Nereis (Neanthes) arenaceodentata* exposed to the Sequim control sediment accumulated significant amounts of cadmium (0.67 mg/kg dry weight) and lead (1.89 mg/kg dry weight).
- Comparisons of tissue residue data with bulk sediment chemistry suggest very little bioaccumulation of contaminants from San Francisco Bay sediment by *N. arenaceodentata*.
- Minimal bioaccumulation may account for minimal toxicity observed in previous chronic sublethal toxicity tests with *N. arenaceodentata*.

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**Table 1**  
**Milestones in the Regulation of Dredged Material in San Francisco Bay**

1965	Committee on Tidal Hydraulics suggests San Francisco District (CESPN) may be dredging a significant amount of material.
1970	Passage of the National Environmental Policy Act.
1970	CESPN initiates Dredge Disposal Study. Terminated in 1975.
1972	CESPN reduces the number of in-bay disposal sites from 11 to 5.
1972	California RWQCB adopts USEPA's Jensen bulk sediment criteria. Material classified as "polluted" by these criteria was either placed upland or taken offshore to the 180-m ocean disposal site.
1973	USACE initiates Dredged Material Research Program.
1976	USACE publishes interim guidance manual for implementation of section 404 (b) of Public Law 92-500.
1977	Publication of USEPA/USACE Ocean Disposal Implementation Manual.
1978	Public Notice 78-1 (PN 78-1) was drafted by the CESPN. Elutriate test procedures adopted from the Ocean Disposal Implementation Manual and in-bay disposal limited to three dispersive sites (Alcatraz, San Pablo Bay, and Carquinez Strait).
1980	California RWQCB adopts PN 78-1.
1980	100-fathom ocean disposal site becomes part of the Point Reyes-Farallon Islands Marine Sanctuary and is subsequently removed from the final designation process by USEPA.
1982	Mounding at the Alcatraz site noted in November.
1984	CESPN implements slurry policy to enhance dispersion during disposal.
1985	CESPN establishes the Disposal Management Program to find operational, environmentally acceptable solutions to disposal problems.
1985	San Francisco Bar Channel ocean disposal site receives final designation by USEPA. It can receive only coarse-grained material.
1988	Bioassay procedures used to evaluate Oakland Inner Harbor sediments under section 401 of the Clean Water Act.
1989	The Long-Term Management Strategy was initiated to reflect increasing regulatory and environmental concerns related to dredged material disposal in San Francisco Bay.
1991	Final revision of USEPA/USACE Ocean Disposal Implementation Manual.

**Table 2**  
**Milestones in Scientific Development of Sediment Toxicity Tests**

1971	Gannon and Beeton published first journal article on sediment bioassays.
1973	USACE initiates Dredged Material Research Program (DMRP).
1976	Publication of Priority Pollutant List by USEPA.
1976	Publication of USACE 404 manual.
1977	Publication of USEPA/USACE Ocean Disposal Implementation Manual.
1978	DMRP completed.
1984	Pellston Conference on Fate and Effect of Sediment-Bound Chemicals.
1987	Formation of ASTM Subcommittee E47.03 on Sediment Toxicology.
1991	Final revision of USEPA/USACE Ocean Disposal Implementation Manual.

**Table 3**  
**Mean (SE) Physicochemical Characteristics of Test Sediments and Pore Water**

	Test Sediment	
	Sequim Control	Oakland Contaminated
Sediment		
Sand, %	13.0* (0.0)	19.2 (0.7)
Silt, %	40.0* (0.0)	53.3 (0.7)
Clay, %	47.0* (0.0)	27.5 (1.6)
Moisture, %	64.7 (1.1)	51.0* (0.0)
LOI, %	14.0 (0.2)	11.8* (0.2)
TOC, %	0.69 (0.11)	0.21* (0.06)
TKN, mg/kg	3,540 (316)	533* (53)
Pore Water		
Salinity, ppt	32.0* (0.0)	32.0* (0.0)
H <sub>2</sub> S, mg/L	133 (27)	<0.01*
NH <sub>3</sub> , mg/L	13.7 (1.5)	42.2* (0.1)
Note: LOI = Loss on ignition, TOC = Total organic carbon, TKN = Total Kjeldahl nitrogen, H <sub>2</sub> S = hydrogen sulfide, NH <sub>3</sub> = total ammonia, * = significantly different (p < 0.05), N = 3. * Lack of variance precluded statistical comparison.		

**Table 4**  
**Mean (SE) Metal Concentrations (mg/kg dry weight) in Test Sediments**

Metal	Test Sediment	
	Sequim Control	Oakland Contaminated
Arsenic	9.16 (0.158)	9.63 (0.166)
Cadmium	0.90 (0.012)	1.01 (0.006)
Chromium	45.9 (0.83)	229* (4.5)
Copper	35.2 (1.80)	133* (2.8)
Lead	26.3 (1.45)	122* (16.8)
Mercury	<dl	4.06 (0.034)
Nickel	42.0 (1.14)	142* (18.9)
Selenium	0.79 (0.017)	0.35* (0.009)
Silver	0.22 (0.012)	0.74* (0.012)
Zinc	83.1 (2.55)	267* (4.4)
Monobutyltin	0.017 (0.0000)	0.072* (0.0010)
Dibutyltin	0.007 (0.0000)	0.177* (0.0080)
Tributyltin	0.006 (0.0010)	0.257* (0.0140)
Note: * = significantly different (p < 0.05), N = 3, <dl = below detection limit.		

**Table 5**  
**Mean (SE) Polyaromatic Hydrocarbon Concentrations (mg/kg dry weight) in Test Sediments**

PAH	Test Sediment	
	Sequim Control	Oakland Contaminated
Naphthalene	< dl	< dl
Acenaphthylene	< dl	< dl
Fluorene	< dl	< dl
Phenanthrene	< dl	38.0 (1.53)
Anthracene	< dl	< dl
Fluoranthene	< dl	38.9 (2.94)
Pyrene	< dl	26.9 (1.21)
Chrysene	< dl	10.5 (0.62)
Benzo(a)Anthracene	< dl	6.93 (0.393)
Benzo(b)Fluoranthene	< dl	10.3 (0.61)
Benzo(k)Fluoranthene	< dl	< dl
Benzo(a)Pyrene	< dl	12.5 (0.79)
Indeno(1,2,3-c,d)Pyrene	< dl	13.3 (0.34)
Dibenzo(a,h)Anthracene	< dl	< dl
Benzo(g,h,i)Perylene	< dl	12.3 (1.07)
Note: N = 3, < dl = below detection limit.		

**Table 6**  
**Mean (SE) Pesticide and Polychlorinated Biphenyl Concentrations**  
**(PCBs) (mg/kg dry weight) in Test Sediments**

	Test Sediment	
	Sequim Control	Oakland Contaminated
Pesticides		
Aldrin	< dl	< dl
$\alpha$ -BHC	< dl	< dl
$\beta$ -BHC	< dl	< dl
$\gamma$ -BHC	< dl	< dl
$\delta$ -BHC	< dl	< dl
p,p'-DDD	< dl	< dl
p,p'-DDE	< dl	0.039 (0.0000)*
Heptachlor	< dl	< dl
Dieldrin	< dl	< dl
Endosulfan I	< dl	< dl
Endosulfan II	< dl	< dl
Endosulfan Sulfate	< dl	< dl
Endrin Aldehyde	< dl	< dl
Heptachlor Epoxide	< dl	< dl
Methoxychlor	< dl	< dl
Toxaphene	< dl	< dl
$\alpha$ -Chlordane	< dl	< dl
$\gamma$ -Chlordane	< dl	< dl
PCBs		
PCB-1016	< dl	< dl
PCB-1232	< dl	< dl
PCB-1242	< dl	< dl
PCB-1248	< dl	< dl
PCB-1254	< dl	< dl
PCB-1260	< dl	< dl
Note: N = 3, <dl = below detection limit. * Lack of variance precluded statistical comparison.		



**Table 7**  
**Mean (SE) Metal Concentrations (mg/kg dry weight) in Worm Tissue**

Metal	Test Sediment	
	Sequim Control	Oakland Contaminated
Arsenic	0.06 (0.009)	0.67 (0.325)
Cadmium	0.67 (0.035)	0.07* (0.010)
Chromium	4.23 (0.650)	2.44 (1.51)
Copper	10.5 (1.31)	10.9 (0.74)
Lead	1.89 (0.545)	0.09* (0.041)
Mercury	<dl	<dl
Nickel	4.17 (0.535)	4.72 (0.631)
Selenium	<dl	<dl
Silver	0.06 (0.009)	0.30* (0.055)
Zinc	34.7 (2.57)	26.1 (2.07)
Monobutyltin	<dl	<dl
Dibutyltin	<dl	<dl
Tributyltin	0.005 (0.0000)*	0.298 (0.0066)

Note: \* = significantly different ( $p < 0.05$ ),  $N = 3$ , <dl = below detection limit.  
 \* Lack of variance precluded statistical comparison.

**Table 8**  
**Mean (SE) Polyaromatic Hydrocarbon Concentrations (mg/kg dry weight) in Worm Tissue**

PAH	Test Sediment	
	Sequim Control	Oakland Contaminated
Naphthalene	< dl	< dl
Acenaphthylene	< dl	< dl
Fluorene	< dl	< dl
Phenanthrene	< dl	< dl
Anthracene	< dl	< dl
Fluoranthene	< dl	< dl
Pyrene	< dl	< dl
Chrysene	< dl	< dl
Benzo(a)Anthracene	< dl	< dl
Benzo(b)Fluoranthene	< dl	< dl
Benzo(k)Fluoranthene	< dl	< dl
Benzo(a)Pyrene	< dl	< dl
Indeno(1,2,3-c,d)Pyrene	< dl	< dl
Dibenzo(a,h)Anthracene	< dl	< dl
Benzo(g,h,i)Perylene	< dl	< dl
Note: Samples were pooled. <dl = below detection limit.		

**Table 9**  
**Mean (SE) Pesticide and Polychlorinated Biphenyl Concentrations**  
**(PCBs) (mg/kg dry weight) in Test Sediments**

	Test Sediment	
	Sequim Control	Oakland Contaminated
<b>Pesticides</b>		
Aldrin	<dl	0.016
$\alpha$ -BHC	<dl	<dl
$\beta$ -BHC	<dl	<dl
$\gamma$ -BHC	<dl	<dl
$\delta$ -BHC	0.047	<dl
p,p'-DDD	<dl	<dl
p,p'-DDE	<dl	<dl
Heptachlor	<dl	<dl
Dieldrin	0.021	0.021
Endosulfan I	<dl	<dl
Endosulfan II	<dl	<dl
Endosulfan Sulfate	<dl	<dl
Endrin Aldehyde	<dl	<dl
Heptachlor Epoxide	<dl	<dl
Methoxychlor	<dl	<dl
Toxaphene	<dl	<dl
$\alpha$ -Chlordane	<dl	<dl
$\gamma$ -Chlordane	<dl	<dl
<b>PCBs</b>		
PCB-1016	<dl	<dl
PCB-1232	<dl	<dl
PCB-1242	<dl	<dl
PCB-1248	<dl	<dl
PCB-1254	<dl	<dl
PCB-1260	<dl	<dl
Note: Samples were pooled. <dl = below detection limit.		

# **Appendix A**

## **Sediment Sampling Locations**

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SEDIMENT SAMPLE <sup>a</sup>	SAMPLING STATION <sup>b</sup>	DATE SAMPLED	LATITUDE/LONGITUDE COORDINATES	
			NORTH (Y)	EAST (X)
SC	SEQUIM	09-OCT-90	49° 03.68'	123° 01.33'

<sup>a</sup> WES sample designation (see Material and Methods).

<sup>b</sup> Battelle site designation.

SEDIMENT SAMPLE <sup>a</sup>	SAMPLING STATION <sup>b</sup>	DATE SAMPLED	CALIFORNIA STATE PLANE COORDINATES (ZONE III)	
			NORTH (Y)	EAST (X)
OC	I-M-1	09-OCT-90	476363	1485762
OC	I-T-6	09-OCT-90	475357	1483653

<sup>a</sup> WES sample designation (see Material and Methods).

<sup>b</sup> Battelle site designation.

# **Appendix B**

## **Water Quality Parameter**

### **Monitoring**

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# WATER QUALITY

WATER QUALITY PARAMETERS DURING 9 WEEKS OF EXPOSURE  
TO BEDDED SAN FRANCISCO BAY SEDIMENTS MEAN (SE) (N=8).

SEDIMENT		TOTAL				
SAMPLE	REP	TEMP. (°C)	SAL. (ppt)	D.O. (mg/L)	pH	NH <sub>3</sub> (mg/L)
=====						
SC	1	19	31	7.8	8.13	0.23
		(0.7)	(0.2)	(0.2)	(0.03)	(0.05)
SC	2	19	31	7.9	8.15	0.14
		(0.7)	(0.2)	(0.2)	(0.03)	(0.09)
SC	3	19	32	7.7	8.14	0.21
		(0.7)	(0.3)	(0.1)	(0.04)	(0.03)
OC	1	18	31	7.9	8.27	0.16
		(0.7)	(0.2)	(0.2)	(0.03)	(0.01)
OC	2	18	32	8.0	8.26	0.14
		(0.7)	(0.3)	(0.2)	(0.03)	(0.02)
OC	3	18	32	7.8	8.29	0.16
		(0.7)	(0.4)	(0.2)	(0.04)	(0.05)

# WATER QUALITY

## WATER QUALITY PARAMETERS DURING REPRODUCTIVE MONITORING, MEAN (SE) (N = 55).

SEDIMENT SAMPLE	TREATMENT	TEMP.(°C)	SAL. (ppt)	D.O. (mg/L)	pH	TOTAL NH <sub>3</sub> (mg/L)
SC	0.25X	20.2 (0.07)	30.1 (0.07)	7.28 (0.069)	7.95 (0.014)	0.13 (0.029)
SC	0.50X	20.2 (0.07)	30.1 (0.07)	7.32 (0.065)	7.97 (0.011)	0.10 (0.033)
SC	1.00X	20.2 (0.08)	30.0 (0.06)	7.36 (0.076)	7.92 (0.012)	0.27 (0.064)
SC	2.00X	20.2 (0.08)	29.9 (0.07)	7.37 (0.099)	7.92 (0.016)	0.37 (0.089)
OC	0.25X	20.2 (0.06)	30.1 (0.07)	7.93 (0.014)	7.94 (0.014)	0.09 (0.026)
OC	0.50X	20.2 (0.07)	30.0 (0.07)	7.37 (0.081)	7.98 (0.017)	0.21 (0.064)
OC	1.00X	20.2 (0.08)	30.0 (0.07)	7.31 (0.092)	7.95 (0.013)	0.43 (0.073)
OC	2.00X	20.2 (0.08)	30.0 (0.07)	7.44 (0.07)	7.93 (0.010)	0.43 (0.079)



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6. AUTHOR(S) David W. Moore Thomas M. Dillon				
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13. ABSTRACT (Maximum 200 words)  <p>In previous studies with San Francisco Bay sediments, minimal chronic sublethal effects were detected (Miscellaneous Paper D-93-1 and another Miscellaneous Paper in preparation by Moore and Dillon). To ensure that the lack of effects was not due to a lack of contaminant uptake, a bioaccumulation experiment was conducted. Bioaccumulation from bedded sediments was evaluated following a 9-week exposure with the marine polychaete worm <i>Nereis (Neanthes) arenaceodentata</i>. Two sediments were evaluated, a contaminated San Francisco Bay test sediment and a "clean" control sediment from Sequim, WA.</p> <p>Animals were exposed as early juveniles through adulthood. Tissues were analyzed for metals, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pesticides. Worms exposed to the contaminated San Francisco Bay sediment had significantly higher tissue residues of silver (0.30 mg/kg dry weight) and tributyltin (0.298 mg/kg dry weight). Conversely, tissue residues of control animals were significantly higher in cadmium (0.67 mg/kg dry weight) and lead (1.89 mg/kg dry weight). Small amounts (0.02 mg/kg dry weight) of aldrin and dieldrin were measured in worms exposed to the contaminated sediment, while dieldrin and <math>\delta</math>-BHC were found in</p>				
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control animals (0.02 and 0.05 mg/kg dry weight, respectively). No PAHs or PCBs were detected in worm tissue from either sediment. Comparisons with bulk chemistry data suggest very little bioaccumulation of contaminants by *N. arenaceodentata*. Minimal bioaccumulation probably accounts for the minimal toxicity observed in previous studies.